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Effects of ACTH and Cortisone on the Pituitary, Thyroid and Gonads of the Teleost *Astyanax mexicanus*[1]

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(Plates I & II; Text-figures 1 & 2)

ALTHOUGH considerable work has been done on the effects of ACTH and cortisone on mammals, comparatively little attention has been given cold-blooded vertebrates. Much of the mammalian histological work has been limited to a study of one or two different organs under identical experimental procedures. The use of a small teleost, *Astyanax mexicanus* (Filippi), affords an opportunity to study the action of administered hormones on several affected organs simultaneously because it is practicable to section the entire fish serially.

Rasquin (1951) and Rasquin & Hafter (1951a), in experiments dealing with adrenal cortical function in normal and tumorous teleosts, found evidence tending to show a depression of thyroid activity and some inconclusive evidence concerning pituitary function after administration of ACTH. The present experiment was designed to clarify these results and to provide information on the effects of continuous injection of ACTH and cortisone on both these glands as well as the gonads, and to discover whether the effects were reversible.

MATERIALS AND METHODS

Ninety-six sexually mature fish, *Astyanax mexicanus* (Filippi), of the same spawning, were divided into six groups of 16 fish each and were treated as shown in the schedule of Table 1. Four groups received daily doses respectively of ACTH, two different doses of cortisone acetate and control injections of physiological saline. Each hormone dose was given in 0.06cc of 0.6% saline; controls received 0.06cc of 0.6% saline. Daily doses of hormone for these groups were 0.2mg cortisone acetate, 0.05mg cortisone acetate and 0.1mg ACTH. Two groups received identical doses of ACTH and physiological saline divided into two injections per day. The ACTH was given as 0.05mg in 0.03cc saline, corresponding to the volume given to the controls. The double daily injections were considered necessary because other investigators have demonstrated that ACTH is rapidly inactivated in the body. Van Dyke, Simpson, Li & Evans (1950) have shown that the life of the ACTH molecule in the circulation is extremely short.

The cortisone acetate was received as a saline suspension. Inasmuch as this was mammalian physiological saline, the suspension was diluted with distilled water from 0.9% to 0.6% for use in fish. To make up the 0.05mg doses, the suspension was further diluted with 0.6% saline so that each dose would be contained in 0.06cc, equivalent in fluid volume to those dosages given to the other fishes injected once per day.

Injections were made for ten consecutive days and two fish of each group were preserved on the third, fifth, eighth and tenth days after the beginning of the injection period and at similar times after the termination of injections. The animals were killed four hours after their final injection, allowing sufficient time to elapse for any shock effect to be obliterated. Two hours

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TABLE 1. MODIFICATIONS OF THYROID AND PITUITARY INDUCED BY ACTH AND CORTISONE

Fish No. and Sex	Weight in grams	Total Dosage in mgms.	*Thyroid* Mean Thyroid Height in microns	*Thyroid* Standard Error	*Pituitary* Total No. Cells in Transitional Lobe	*Pituitary* % Basophils	*Pituitary* % Acidophils	*Pituitary* % Chromophobes
Controls (One Injection per Day)								
1-M	1.3	———	3.13	0.0802	5,067	59.4	38.4	2.2
2-F	2.3	———	3.23	0.0819	15,071	46.0	52.5	1.5
3-M	1.1	———	2.78	0.0596	6,671	52.0	45.9	2.1
4-F	2.7	———	3.38	0.0712	25,389	67.6	31.3	1.1
5-M	2.0	———	3.36	0.0713	15,236	63.6	35.5	1.9
6-F	1.4	———	3.58	0.0854	7,482	49.0	48.2	2.8
7-M	2.0	———	2.94	0.0713	13,510	60.4	38.2	1.4
8-F	1.6	———	2.83	0.0701	10,963	56.9	40.8	2.3
9-M	1.3	———	3.20	0.0711	7,970	51.5	47.0	1.5
10-F	1.5	———	3.13	0.0702	7,914	61.6	36.9	1.5
11-M	0.8	———	3.13	0.0798	6,800	62.8	34.3	2.9
12-F	3.2	———	2.96	0.0644	14,674	62.0	36.9	1.1
13-M	0.9	———	3.11	0.0675	5,258	52.2	45.4	2.4
14-F	2.5	———	3.14	0.0734	18,016	52.2	46.9	0.9
15-M	2.3	———	2.95	0.0563	18,494	51.9	47.0	1.1
16-F	1.4	———	3.10	0.0686	12,506	61.5	37.3	1.2
Cortisone-injected								
17-M	1.9	0.6	2.18	0.0581	7,624	44.3	54.0	1.3
18-F	2.9	0.6	2.78	0.0810	12,729	47.0	51.3	1.7
19-M	2.5	1.0	2.44	0.0619	15,350	54.9	44.3	0.8
20-F	1.7	1.0	2.79	0.0888	11,700	46.1	51.8	2.1
21-M	2.1	1.6	2.58	0.0639	9,471	49.8	49.2	1.0
22-F	1.8	1.6	2.51	0.0541	11,756	42.2	55.9	1.9
23-M	2.1	2.0	2.52	0.0628	16,098	38.8	60.8	0.4
24-F	1.5	2.0	2.56	0.0611	8,698	44.2	54.2	1.6
25-M	2.1	———	2.17	0.0549	12,565	37.9	61.0	1.1
26-M	1.3	———	2.23	0.0526	7,626	42.2	57.0	0.8
27-M	1.3	———	2.55	0.0545	7,865	46.2	53.2	0.6
28-M	1.4	———	2.55	0.0656	5,405	41.6	57.3	1.1
29-M	2.2	———	2.65	0.0670	11,151	50.5	48.5	1.0
30-M	1.4	———	2.52	0.0680	8,015	25.1	73.3	1.6
31-M	1.8	———	2.63	0.0698	13,818	40.4	58.5	1.1
32-M	1.5	———	2.41	0.0618	16,122	40.2	57.8	2.0
33-M	2.7	0.15	2.08	0.0487	13,188	65.5	33.5	1.0
34-F	3.3	0.15	2.60	0.0626	30,997	44.2	55.2	0.6
35-M	1.3	0.25	2.36	0.0584	9,774	44.5	54.6	0.9
36-M	2.1	0.25	2.73	0.0634	11,374	49.2	49.8	1.0
37-M	1.8	0.40	2.48	0.0560	6,580	49.8	49.5	0.7
38-F	2.0	0.40	2.59	0.0661	11,334	55.9	43.0	1.1
39-M	1.5	0.50	2.18	0.0526	10,136	55.7	43.1	1.2
40-M	1.3	0.50	2.28	0.0580	7,245	49.2	49.7	1.1
41-M	1.4	———	2.39	0.0618	10,877	44.6	54.3	1.1
42-M	1.3	———	2.54	0.0658	9,320	39.9	58.8	1.3
43-M	0.8	———	2.62	0.0640	4,915	50.6	48.2	1.2
44-M	3.1	———	2.75	0.0743	14,947	51.4	48.0	0.6
45-M	1.9	———	2.38	0.0596	14,579	47.3	52.0	0.7
46-M	1.1	———	2.77	0.0664	5,870	46.3	53.0	0.7
47-M	1.6	———	2.77	0.0779	8,994	45.6	53.4	1.0
48-M	1.3	———	2.85	0.0697	9,191	51.6	47.3	1.1

TABLE 1. MODIFICATIONS OF THYROID AND PITUITARY INDUCED BY ACTH AND CORTISONE (CONTINUED)

Fish No. and Sex	Weight in grams	Total Dosage in mgms.	*Thyroid* Mean Thyroid Height in microns	*Thyroid* Standard Error	*Pituitary* Total No. Cells in Transitional Lobe	*Pituitary* % Basophils	*Pituitary* % Acidophils	*Pituitary* % Chromophobes
ACTH-injected								
49-M	1.5	0.3	2.45	0.0680	6,562	45.6	53.0	1.4
50-F	2.5	0.3	2.81	0.0751	13,666	64.2	35.0	0.8
51-M	2.2	0.5	2.30	0.0557	16,766	50.7	47.4	1.9
52-F	2.6	0.5	2.67	0.0767	10,575	52.2	46.0	1.8
53-M	1.5	0.8	2.47	0.0595	5,356	48.8	48.3	2.9
54-F	1.7	0.8	2.35	0.0589	8,100	52.0	45.5	2.5
55-M	1.6	1.0	2.37	0.0560	11,579	40.6	57.7	1.7
56-F	1.5	1.0	2.17	0.0549	14,254	57.1	41.8	1.1
57-M	1.9	———	2.24	0.0626	14,383	43.2	55.2	1.6
58-F	2.5	———	2.37	0.0626	12,928	58.0	40.9	1.1
59-M	1.6	———	2.58	0.0642	9,373	46.0	51.7	2.3
60-F	2.6	———	2.87	0.0757	20,663	56.6	41.8	1.6
61-M	1.7	———	2.80	0.0819	6,776	48.9	49.8	1.6
62-M	1.1	———	2.98	0.0749	5,612	62.4	36.2	1.4
63-M	1.7	———	3.06	0.0734	10,025	52.3	46.6	1.1
64-M	1.3	———	3.10	0.0777	8,599	62.8	36.5	0.7
65-M	1.9	0.3	2.25	0.0559	10,969	66.1	33.7	0.2
66-F	1.7	0.3	2.42	0.0541	13,220	61.0	38.0	1.0
67-M	1.2	0.5	2.22	0.0558	7,396	54.7	44.5	0.8
68-F	2.8	0.5	2.10	0.0488	15,512	57.3	41.7	1.0
69-M	1.4	0.8	2.33	0.0602	9,520	40.1	59.0	0.9
70-F	1.9	0.8	2.18	0.0481	11,524	50.7	48.4	0.9
71-M	1.2	1.0	2.33	0.0580	8,853	39.3	59.2	1.5
72-F	3.0	1.0	2.27	0.0545	23,096	50.3	47.9	1.8
73-M	1.8	———	2.34	0.0599	21,710	43.1	55.5	1.4
74-F	1.8	———	2.30	0.0587	10,498	51.3	47.7	1.0
75-M	1.8	———	2.14	0.0514	15,738	52.9	46.1	1.0
76-F	1.8	———	2.92	0.0783	13,902	55.1	43.4	1.5
Controls (Two Injections per Day)								
77-M	1.8	———	2.57	0.0623	14,603	52.2	45.7	2.1
78-F	3.2	———	2.49	0.0620	19,825	74.5	24.3	1.2
79-M	1.8	———	2.65	0.0634	13,465	53.2	45.6	1.2
80-F	2.7	———	2.88	0.0678	19,506	57.6	41.5	2.9
81-M	1.6	———	2.72	0.0613	12,028	56.3	42.7	1.0
82-F	1.3	———	2.72	0.0915	17,831	50.1	47.8	2.1
83-M	1.3	———	3.03	0.0700	8,579	46.1	52.8	1.1
84-F	1.8	———	2.46	0.0629	13,329	55.8	43.1	1.1
85-M	2.4	———	2.96	0.0682	14,183	51.3	47.7	1.0
86-F	2.9	———	2.85	0.0754	17,575	64.3	34.8	0.9
87-M	1.8	———	3.13	0.0712	13,100	58.6	40.1	1.3
88-F	1.5	———	3.07	0.0703	12,369	61.0	37.9	1.1

after injection with ACTH, shock effects were observed, but no such effects were noted four hours after injection (Rasquin, 1951). An attempt was made to use equal numbers of males and females and to sacrifice one of each sex at each experimental period. Actually more males than females were selected.

The fish were killed by immersion in Bouin's fluid. The gonads and the part of the kidney containing the corpuscles of Stannius were removed

and sectioned separately. The remainder of each fish after decalcification was serially sectioned transversely at seven microns from the region of the eye posteriorly through the head-kidney. Sections through the regions of the pituitary were cut at five microns and stained by Masson's trichrome method. The other sections were stained with either Masson's trichrome stain or Harris's hematoxylin and eosin. The term "acidophil" as used here refers to those cells that stain with acid fuchsin or ponceau, and "basophil" denotes those cells that stain with fast green. Differential cell counts of the transitional lobes of the pituitaries were made by means of a Leitz grid ocular micrometer. All the cells were counted in every fourth section from the beginning to the end of transitional lobe tissue and thus every part of the lobe was represented in the final count. The total number of cells for each fish seen in Table 1 is therefore an index and is not intended to represent the actual numbers of cells. Thyroid epithelial heights were measured after the method of Rawson & Starr (1938), except that 100 instead of 200 measurements were taken on each fish.

Five times a week the animals were fed a prepared dried food containing dried shrimp, both muscle and shell, liver, chopped lettuce and spinach, Pablum and salt. The formula for making this food has been published by Aronson (1949).

Experimental Results

In the transitional lobes of the pituitaries of *Astyanax mexicanus* the basophils number approximately 60% of the total cells and the acidophils number about 40% (Rasquin, 1949). Chromophobes are relatively scarce, making up only 1% or 2%, rarely 3%, of the total. The normal percentage of basophils was lowered by all the injection schedules, including those of the saline used for controls. These results differ from those of Golden & Bondy (1952), who found that in rats cortisone caused an increase in basophil count, while ACTH evoked no significant change in the count.

All the data used for making the graphs and for statistical analysis may be found in Table 1. The table also gives the total dosages of hormone received by each injected fish, and comparisons may be made between the states of the thyroid and pituitary glands of any individuals.

Text-figure 1 illustrates graphically the effects of ACTH and cortisone injection on the percentage of pituitary basophils. Four fish receiving two ACTH injections per day died before the end of the experiment and their corresponding controls were not sectioned. The data indicate that there is a significant difference between the male and female reaction to ACTH. Both series of ACTH injections lowered the proportion of basophils in the males while the proportions of cells in the female pituitaries showed no significant difference from the controls. Graphs A and B show the results of double and single daily injections, respectively, of ACTH on the male compared with the female. In Graph C the mean of the basophil percentages of the two fish given two injections daily of ACTH is compared with the control, and Graph D is similarly plotted for the single daily injection. Both these graphs show a decrease in the proportion of basophils in the ACTH-injected fish when compared with the saline-injected controls. There is no significant difference in effect between the double and single daily injections. In both groups of fish a tendency to return to the normal ratio is seen after the tenth day when injections ceased.

Graphs E and F of Text-figure 1 show the results of large and small doses of cortisone respectively on the males compared with the females. Cortisone lowers the proportion of basophils in both males and females and there is no significant difference in the reactions of the two sexes. Only males were left in these groups of fish after injections were completed. In graphs G and H the means of basophil percentages of the two fish given large and small doses of cortisone respectively have been compared with the controls. In both instances there is a decrease in the proportion of basophils, although the larger dose is more effective than the smaller. The statistical analysis indicates that no trend to recovery within the post-injection period occurred in the pituitaries of the cortisone-treated fish. Histologically, however, some recovery was evidenced by the appearance of mitotic figures in basophils.

The inversion of the normal basophil-acidophil ratio is accompanied by profound histological changes. Plate I, Figure 1, is a photomicrograph of a part of the transitional region of one of the single daily injected controls. This gland is not different histologically from the normal in which most of the basophils show some vacuolation. Vacuolation is definitely increased in the basophils of the controls receiving two saline injections per day and some degranulation also occurs so that the staining reaction is weaker (Figure 2). The acidophils, which are the smaller, dark, polygonal cells, appear to be unaffected by the saline injection routines. Basophils of the ACTH-injected fish show no loss of vacuolation but no degranulation was evident. The cytoplasm maintains its intense staining affinity, although pycnotic nuclei are sometimes seen, particularly near the periphery of the

gland. Basophils of the cortisone-treated fish frequently give evidence of complete exhaustion, with a loss of normal staining reaction and large vacuoles filled with debris. Mitotic figures are occasionally seen in the basophils of this group and these are most frequent in the fish killed in the post-injection period. Plate I, Figure 3, shows a basophil in anaphase in the transitional lobe of a fish killed eight days after cortisone injections began. In both ACTH- and cortisone-injected fish there is a hypertrophy and an increase in numbers of acidophils. The same phenomenon is observed in both groups but is more pronounced in the cortisone-treated fish where the condition is maintained to the end of the post-injection period. The acidophils increase greatly in size of nucleus and quantity of cytoplasm and are so filled with secretion and so intensely stained that the cell boundaries are often impossible to locate and the tissue has become friable like colloid. Plate I, Figure 4, shows the hypertrophy and hyperplasia of acidophils in a cortisone-treated fish. The indication is that the acidophils are witholding their secretion, and that the injected hormones have in some way interfered with its normal release.

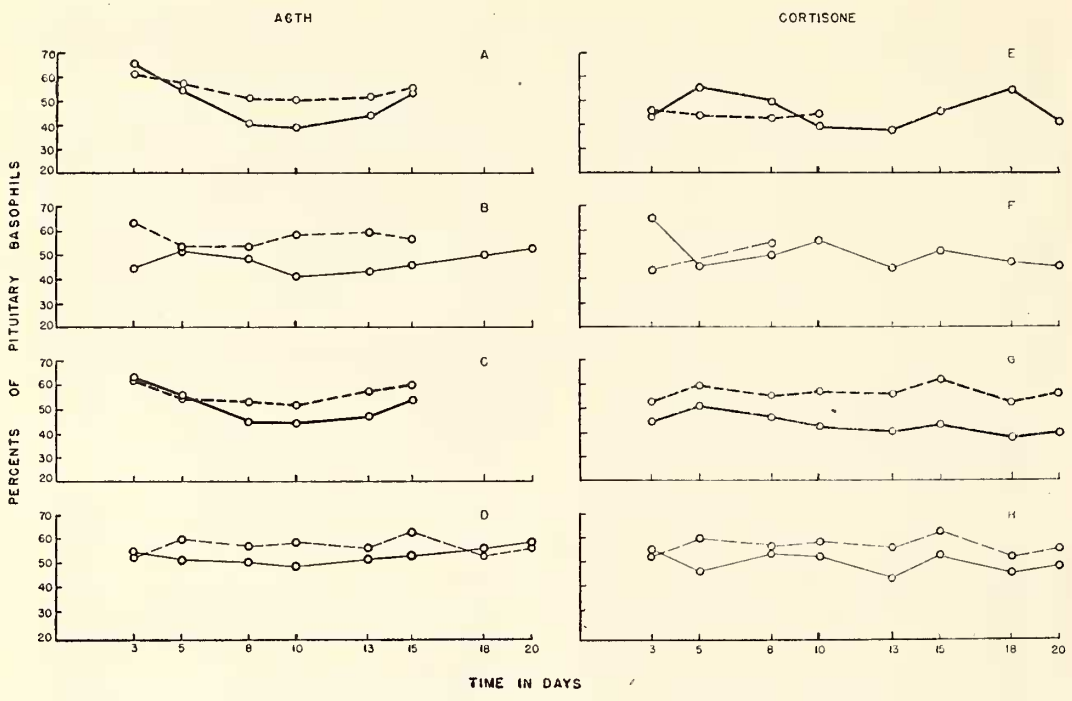
The cause of the inversion in the ratio of acidophils to basophils in the treated fish is not clear. In all the pituitaries examined only two mitotic figures were seen in acidophils. It seems probable that most of the new acidophils come from the granulation of chromophobes, which also are occasionally seen in mitosis and often appear with a few acidophilic granules in the cytoplasm. These latter sparsely granulated cells were counted as acidophils. The decrease in ratio of basophils is not merely a numerical function of the increase of acidophils alone, for this decrease is seen to some extent in the controls where histologically the acidophils show no change from the normal. There is no indication that basophils are changed into any other type of cell. There is no evidence of necrosis in any of these glands nor is any phagocytosis seen. Occasionally lymphocytes are observed in the glands but these do not appear to be engaged in phagocytic activity.

It is possible that the technique of counting is responsible for what may be only an apparent loss of basophils. In order that no duplication might occur, every fourth section was counted, because the sections were five microns thick and some of the active basophils measured nearly 20 microns across. However, clusters of nuclei of basophils practically devoid of cytoplasm were seen in many of the pituitaries and many of these would remain uncounted as they would lie between the selected sections. The technique then gives a reliable index only of the functioning basophils. Increasing numbers of basophils in the recovering gland may have their source in regranulation of the depleted cells as well as in mitotic division of more active ones.

Thyroid.—Text-figure 2A is a graphic representation of the effects of ACTH on thyroid epithelial heights. No sex difference is shown in the reaction of the thyroids to either ACTH or cortisone, and therefore the two fish from each experimental period were grouped together and their means compared with those of the two controls. From the graph it can be seen that double daily injections of saline depress the thyroid height below that of single daily injections. Both ACTH injection series depress the thyroid height below that of the controls, the double daily dose being quicker acting and more effective during the experimental period than the single daily injection. After the tenth day when injections ceased, the single daily injected ACTH group shows recovery of the thyroid and indication of a return to normal is observed in the double daily injected ACTH group. Text-figure 2B shows graphically the effects of large and small doses of cortisone on thyroid epithelial heights. While both dosages of cortisone depress the height of the thyroid epithelium below that of the controls, there is no statistically significant difference between the effects of the two doses of cortisone. With neither dosage of cortisone does the height of the thyroid epithelium return to that of the control fish during the post-injection period.

Histologically, the thyroids of the control fish show a low cuboidal epithelium with some vacuoles in both cells and colloid. In both ACTH- and cortisone-treated fish, follicles are larger and the epithelium is so flattened as to be squamous in character in some follicles. It is probable that this effect on the thyroid is not mediated by the pituitary through any loss of thyrotropic function, but is rather a more direct effect of cortical hormones. Table 1 shows that some of the lowest thyroid epithelial heights were associated with pituitaries that were still unimpaired by hormone injections.

Gonads.—The ovaries of all the control fish contain numbers of immature and mature eggs as well as an occasional resorbing egg. The follicular epithelium surrounding the immature egg is flat and becomes higher as the egg matures. In the normal reproductive cycle, the ripe eggs are resorbed if spawning does not take place. The resorption process is carried on by phagocytic activity of infiltrating connective tissue and of the follicular cells which become hyperplastic and hypertrophied at this time. Plate II, Figure 5, is a photomicrograph of the ovary of a saline-injected control showing normal conditions.

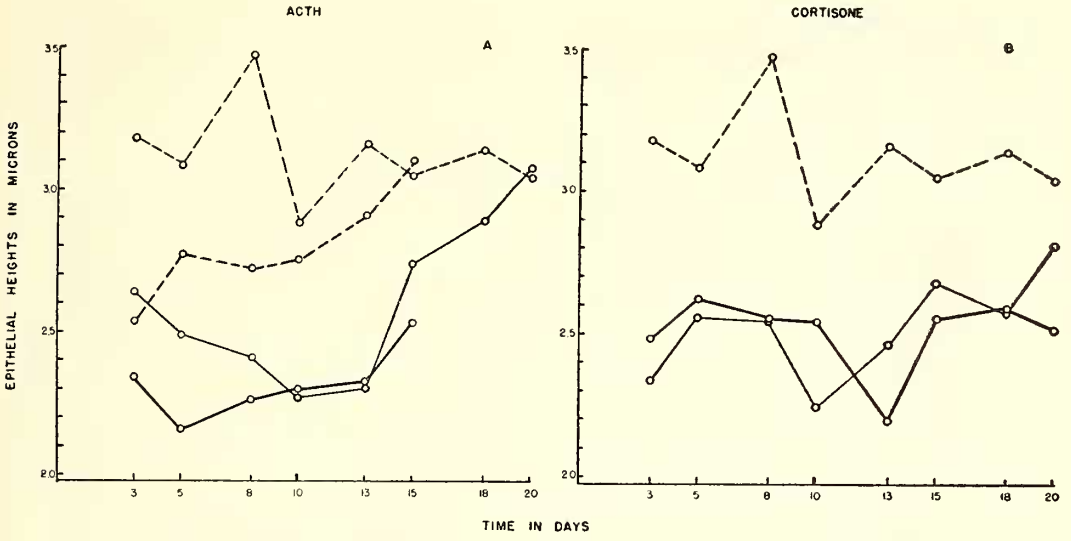


TEXT-FIG. 1. The effect of ACTH and cortisone on percentages of pituitary basophils. In A and B, solid lines indicate the males; broken lines indicate the females, both injected with ACTH. In C and D, solid lines indicate the ACTH-injected fish; broken lines indicate the saline-injected controls. Heavy lines indicate two injections per day; light lines indicate one injection per day. In E and F, solid lines indicate the males; broken lines indicate the females, both injected with cortisone. In G and H, the solid lines indicate the cortisone-injected fish; broken lines indicate the saline-injected controls. Heavy lines indicate the larger dose of cortisone; light lines indicate the smaller dose. Each point of A, B, E and F represents one fish killed at that time. Each point of C, D, G and H represents the mean of two fish killed at that time.

Figure 6 shows the breakdown of the yolk in the mature eggs three days after the start of injections of the larger doses of cortisone. Figure 7 shows the ovary eight days after the start of cortisone injections. Here the tissue seen between the ova is mainly follicular epithelium and remnants of resorbed eggs and some connective tissue. Only mature eggs are resorbed and not all of these are affected. The effects of ACTH given twice daily are comparable with those of cortisone on the ovaries. The effects of one daily injection are of the same nature although of lesser degree.

The testes of all the control animals show cysts of developing spermatogonia and lobules containing mature sperm suspended in fluid secreted by the sperm duct epithelium (Rasquin & Hafter, 1951b). Figure 8 is a photomicrograph of the testis of a control. The effects of the experimental procedures can be seen in Figure 9. Cortisone, and to a lesser extent ACTH, stimulated the secretory activity of the sperm duct epithelium to such an extent that the duct and the lobules were distended with fluid. In such glands there was a decrease in the amount of spermatogenesis. In the normal reproductive cycle the amount of spermatogenesis observed in the testis appears to be correlated with the content of the lobules. In gonads in which the lobules are packed with mature sperm, spermatogenic activity is less than that observed when the lobules are more nearly empty. It is possible therefore that the decreased amount of spermatogenesis observed in the hormone-treated fish is related to the excess fluid within the lobules and not to a direct effect of hormone administration.

Adrenal Cortex and Lymphoid Organs.—The cells of the anterior inter-renal tissue, which is homologous to the mammalian adrenal cortex (Rasquin, 1951), show no degranulation following any of the experimental techniques. When Masson's trichrome staining method is used, the glandular tissue shows fuchsinophile granules, irregular in size and shape in all fishes whether experimental or control. However, both ACTH



TEXT-FIG. 2. The effect of ACTH and cortisone on thyroid epithelial heights. In A, solid lines indicate ACTH-injected fish; broken lines indicate saline-injected controls. Heavy lines indicate two injections per day; light lines indicate one injection per day. In B, solid lines indicate cortisone-injected fish; broken lines indicate saline-injected controls. The heavy line indicates the larger dose of cortisone; the light line indicates the smaller dose. Each point on the graph represents the mean of two fish killed at that time.

injection schedules cause hypertrophy and hyperplasia of the inter-renal tissue. Many mitotic figures were seen in the glandular cells, which spread out from their normal position next to the walls of the cardinal veins into the parenchyma of the head-kidney. One of these dividing cells is seen in Figure 10. After cortisone injections hyperchromatic nuclei are seen in the inter-renal cells and some shrinkage is noted in the glands of the fishes killed early in the experimental period.

The head-kidney is greatly depleted of lymphoid elements after cortisone treatment. Many edematous spaces are evident as well as areas where the only cellular elements remaining are the reticular fibers of the stroma. There is some evidence of repair ten days after the last injection when the lighter dose of cortisone was used, but no recovery has been initiated in the organ at the end of the experimental period when the larger dose of cortisone was used. The administration of ACTH causes a much less drastic reduction in the head-kidney. There is some loss of lymphocytes and appearance of some edematous spaces but this condition is completely repaired ten days after injections were discontinued. The effect is more pronounced and the time before recovery is slightly longer in the fish injected twice a day than in those injected once a day. The lymphoid elements of the head-kidneys of the fish that received the control saline solutions are also somewhat affected, showing in still another way that the control injection schedule also produced a stressful condition. These organs in the control fishes, however, were promptly repaired at the end of the injection period.

All the fish show a partly involuted thymus which is normal for this species at this age (Hafter, 1952). No further involution is noted as a result of any of the experimental procedures. Evidently the thymus, in this species of teleost at least, is extremely refractory to mammalian cortical hormones.

A study of the corpuscles of Stannius, formerly thought to contain part of the teleost cortical tissue, shows no change that can be attributed to any of the experimental procedures. Different degrees of cellular activity were noted and some glands showed a more vascular condition than others but no consistent correlations can be made.

Discussion

An unexpected result of this investigation is the difference in the reaction of the male and female pituitary to ACTH. Explanation of this effect may be provided if it is assumed, first, that the cortical hormone produced by the fish possesses androgenic activity similar to that observed in higher vertebrates; and, second, that in the female, endogenous estrogens antagonize this action so that the total inhibitory effect on the female pituitary is less than that observed

in the male where no such antagonism occurs. No sex difference was observed in the pituitary with either dosage of cortisone, and it seems likely that the mammalian cortical steroid was administered in a large enough dose or was sufficiently androgenic to over-ride any antagonistic action of endogenous estrogens.

The effects of strong masculinizing agents on the teleost gonads have been described by Eversole (1941) who treated male and female guppies, *Lebistes reticulatus*, with testosterone propionate and pregneninolone. Early in the injection period hypertrophy of the testicular ducts and precocious maturation of spermatogonia were produced. New development of spermatogonia, however, was not initiated. Continued injection of these hormones left the testes of *Lebistes* "exhausted and degenerate." As Eversole has also indicated, in the female, ovogenesis and yolk deposition were effected only after prolonged treatment. In the treated mature females the ovaries showed a return to a "juvenile" condition with resorption of yolk and embryos. At the same time, administration of adrenal cortical extracts, of unreported origin, had no effects on the gonads of the same species. In the male *Astyanax*, the administration of ACTH and cortisone brought about the same changes reported by Eversole for his early results in the male guppy. Similarly, in the female *Astyanax*, only already matured ova underwent resorption after treatment; immature eggs were not affected.

Although early workers reported that ACTH stimulated the gonads and accessory sex organs, these results have been contradicted by investigators using purified hormones. Reviews of this material are given by Ingle (1950) and Baker, Schairer, Ingle & Li (1950). From the work of Ingle (1950) and Winter, Silber & Stoerk (1950), it is seen that cortisone administered in small doses causes no significant change in the reproductive system of mature male rats. Massive doses of cortisone cause some regression of the testes and seminal vesicles of mature rats (Ingle, 1950) and mice (Antopol, 1950). Sprague (1951) suggested that the results on the gonads observed with large doses of cortisone may have been a general reflection of the catabolic effects of cortisone rather than a specific effect on the reproductive tract. Similarly, Baker et al. (1950) suggested that a negative nitrogen balance resulting from the metabolic effects of the C-11 oxygenated steroids released from the adrenals after stimulation with ACTH caused the regressive changes observed in the reproductive organs of mature rats after ACTH administration.

The ACTH used for this investigation was contaminated only with small amounts of oxytocin. If contamination by gonadotropins was responsible for the effects on the gonads reported here, the same effects could hardly have been produced by cortisone. It seems most probable that, in this species of teleost, at least, the cortical hormones are androgenic.

However, two other possibilities suggest themselves. Rasquin & Hafter (1951a) have already intimated that the teleost may not react specifically to mammalian ACTH, and perhaps this hormone acted gonadotropically as well as adrenocorticotropically. The second possibility concerns a shift in production of tropic hormones by the pituitary such as has been postulated by Selye (1946) under conditions of stress. The effects of ACTH and cortisone on the *Astyanax* reproductive system may have been caused by an increased production of pituitary gonadotropin associated with an inhibition of adrenocorticotropin production. It is interesting to note here that Witschi (1939) has reported the luteinizing factor to be predominant in teleost gonadotropin. The changes in the ovaries of *Astyanax* were certainly correlated with proliferation of follicular epithelium and the break-down of already matured ova.

Although several investigators (Soffer, Gabrilove & Dorrance, 1951; Money, Kraintz, Fager, Kirschner & Rawson, 1951; Antopol, 1950) have reported that ACTH and cortisone depress the activity of the thyroid in mammals, the mechanism by which this is achieved is not yet known. Money et al. believe that this effect is mediated through the pituitary, while Perry (1951) suggests that the action is a direct one on the thyroid. The results of this report indicate that the effect on the thyroid of *Astyanax* is a direct one. The lowest thyroid epithelial heights, indicative of the least active glands, were not necessarily correlated with pituitaries having the most drastically reduced numbers of basophils. The injection of ACTH twice daily was more effective in reducing thyroid height but was no more effective than one daily injection in changing the pituitary, and conversely, no differences were noted in thyroid response between the two dosages of cortisone, but the pituitaries of the two cortisone groups were statistically different from each other. Were the effect on the thyroid mediated through the pituitary, the responses of the two glands should have been consistent.

Furthermore, there is no histological evidence of a thyrotropic hormone deficiency. In no experimental group was there any evidence of complete exhaustion of all basophils, and it does not seem likely that the acidophils have a thyrotropic function. The pituitary glands of some

of the fishes that showed extremely low thyroid epithelium had not yet arrived at a point where the acidophils were withholding secretion. In addition, although the thyroid epithelium of the controls injected twice per day was more depressed than those of controls injected once per day, the pituitaries of neither control group showed any cytological change in acidophils. Thus there is no consistent correlation between thyroid depression and masses of acidophils with retained secretion.

Because the reciprocal function of the pituitary and adrenal is so well known (Sayers, 1950; Tuchmann-Duplessis, 1950, 1951; Ingle, 1950) and becausc an increase in numbers and secretion of acidophils accompanies ACTH and cortisone administration, it is tempting to assign adrenocorticotropic function to the acidophils in this teleost. Thus the presence of exogenous ACTH or increased levels of cortical steroids would inhibit the release of ACTH from the pituitary. However, that the acidophils do not elaborate ACTH is shown by the following: the basophils alone showed intense vacuolation and depletion as a result of the stress caused by the control saline injections; among the cortisone-treated fishes, mitotic figures and other evidences of recovery were shown by the basophils after injections were discontinued; other types of stress, such as living in darkness (Rasquin, 1949) bring about activation and exhaustion of the basophils.

Li & Evans (1947), Winter, Silber & Stoerk (1950), have shown that in rats there is a fundamental antagonism between the adrenocorticotropic and growth hormones. Winter, Silber & Stoerk have said that the antagonism could be due to suppression of the release of the growth hormone or to suppression of its peripheral action. The acidophils of most vertebrates are generally credited with the elaboration of the growth hormone. If this should be true of the acidophils of the transitional lobe of the teleosts, their massed unreleased secretion after ACTH and cortisone administration would indicate that the seat of this antagonism is in the pituitary itself.

Rasquin & Hafter (1951a) injected a lymphosarcomatous fish and a control with the identical doses of ACTH used for this report. Only the tumorous fish showed inversion of the ratio of secretory cells in the hypophysis. These fish were much older than those used for the present report and their mean body weight was 7.8 grams. From the results obtained here it seems probable that the dose given the larger fishes was insufficient to cause the effect on the pituitary in spite of having an effect on the lymphoid tissues. The inversion of the pituitary ratio in the tumorous fish was probably, therefore, associated in some way with the tumorous condition.

Summary

1. Ninety-six fish (*Astyanax mexicanus* (Filippi)) were divided into six groups. Four groups received daily doses respectively of ACTH, two different amounts of cortisone acetate and control injections of physiological saline. Two groups received identical doses of ACTH and physiological saline divided into two injections per day. Injections were continued for ten days and two fish from each group were killed at similar intervals during the injection period and for ten days thereafter.

2. In the transitional lobes of the pituitaries the normal ratio of approximately 40% acidophils to 60% basophils was inverted by ACTH in the males and in both sexes by cortisone. Both hormones lowered the percentages of basophils below that found in the controls.

3. A tendency to return to the normal ratio of secretory cells was noted statistically during the post-injection period in both ACTH groups, but with neither cortisone group, although some histological changes indicating repair were seen in the cortisone group.

4. ACTH and cortisone depressed the height of the thyroid epithelium. Double daily injections of saline depressed the height of the thyroid epithelium below that found in the single daily injected controls but were not as effective as either hormone.

5. Recovery of the thyroid was noted in the single daily injected ACTH group as well as in both control groups. With neither dosage of cortisone did the height of the thyroid epithelium return to that of the control fish during the post-injection period.

6. ACTH and cortisone stimulated secretory activity of the sperm duct epithelium in the testis; lobules were distended with fluid and spermatogenesis appeared to be depressed. In the ovary, both hormones induced resorption of mature eggs and hypertrophy and hyperplasia of follicular epithelium.

7. Both dosages of cortisone and two doses of ACTH per day were more effective in producing changes in the thyroid and gonads than was the single daily ACTH injection.

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EXPLANATION OF THE PLATES

Plate I.

Fig. 1. Section through the transitional lobe of the pituitary of a control fish, saline-injected once per day. Acidophils are small, dark, polygonal cells; basophils are lighter-staining vacuolated cells. Part of the pars nervosa appears at the bottom of the photograph. All pituitary sections were stained in Masson's trichrome stain. × 850.

Fig. 2. Section through the transitional lobe of the pituitary of a control fish, saline-injected twice daily, showing increased vacuolation caused by the additional stress of the double injection schedule. × 850.

Fig. 3. Section through the transitional lobe of a cortisone-treated fish eight days after injections began. A basophil in anaphase is seen at left center. × 850.

Fig. 4. Section through the transitional lobe of the pituitary of a cortisone-treated fish, showing increased numbers of hypertrophied acidophils. Compare with control in Fig. 1. × 850.

Plate II

Fig. 5. Section of ovary of control fish, showing immature and ripe ova. Hematoxylin and eosin. × 85.

Fig. 6. Section of ovary of a cortisone-treated fish three days after the start of injections, showing regressive changes in the yolk of mature ova. Hematoxylin and eosin. × 85.

Fig. 7. Section of the ovary of a cortisone-treated fish eight days after the start of injections, showing resorbed ova, hyperplastic follicular epithelium and connective tissue among normal ova. Hematoxylin and eosin. × 85.

Fig. 8. Section through the testis of a control fish, showing ducts lined with epithelium and filled with ripe sperm. Cysts of developing sperm in various stages are seen mainly in the upper left quadrant of the photograph. Hematoxylin and eosin. × 85.

Fig. 9. Section through the testis of a cortisone-treated fish, showing increase in seminal fluid and possible depression of spermatogenesis. Hematoxylin and eosin. × 85.

Fig. 10. Section through the inter-renal tissue of an ACTH-treated fish, showing hypertrophy and hyperplasia of the cortical cells. A mitotic figure is seen in the lower right quadrant of the photograph. Hematoxylin and eosin. × 85.